

Keiko KOSUGE* & Hiroshi OKADA**: **Cytotaxonomical studies on *Dichocarpum* (Ranunculaceae) in Japan**

小菅桂子*・岡田 博**: 日本産シロカネソウ属の細胞分類学的研究

The genus *Dichocarpum* W.T. Wang et Hsiao is distributed from temperate to warm zone of East Asia and consists of about 18 species. Many of the species were at first described under *Isopyrum* L., but Wang & Hsiao (1964) separated them as a new genus based on connate carpels, long clawed petals and pedately compound leaves. This genus was classified under the tribe Isopyreae by Tamura (1966) and Wang (1979), because *Dichocarpum*, *Isopyrum* and their allies have common features, i.e., karyotype, petal, carpel and so on. Cytological characters have been considered as the most important criteria for the classification of Ranunculaceae (Langlet 1932, Gregory 1941, Tamura 1966).

Chromosome number of five Japanese species of *Dichocarpum* were reported to be $2n=35$ (Kurita 1954, 1956, 1966, 1967, Okada & Tamura 1979). The related genera in tribe Isopyreae, i.e., *Enemion*, *Isopyrum*, *Leptopyrum*, *Semi-aquilegia*, and *Aquilegia*, have constantly $2n=14$ (see for examples, Langlet 1927, Sorokin 1929, Kurita 1954, Taylor & Mulligan 1968). Therefore, Kurita (1956, 1966) considered that *Dichocarpum* species were at pentaploid level with the basic chromosome number of 7.

Previous cytological reports suggest conspicuous differences between *Dichocarpum* and other genera, for instance, pentaploid with diploid of $x=7$. Is this cytological character, a pentaploid of $x=7$, a reliable criteria for the characterization of genus *Dichocarpum*? Pentaploid species are considered to reproduce offsprings by abnormal systems, such as apomixis. How does *Dichocarpum*, a pentaploid taxon, reproduce gametes? In order to evaluate these questions, the somatic chromosomes and meiotic division of PMCs were observed in all Japanese *Dichocarpum* species.

Materials and methods Fresh materials and their localities are shown in

* Department of Biology, Faculty of Science, Kobe University, Kobe 657. 〒657 神戸市灘区六甲台町. 神戸大学 理学部生物学教室.

** Department of Biology, College of General Education, Osaka University, Osaka 560. 〒560 豊中市待兼山町. 大阪大学 教養部生物学教室.

Tab. 1. Chromosome numbers of *Dichocarpum* in Japan.

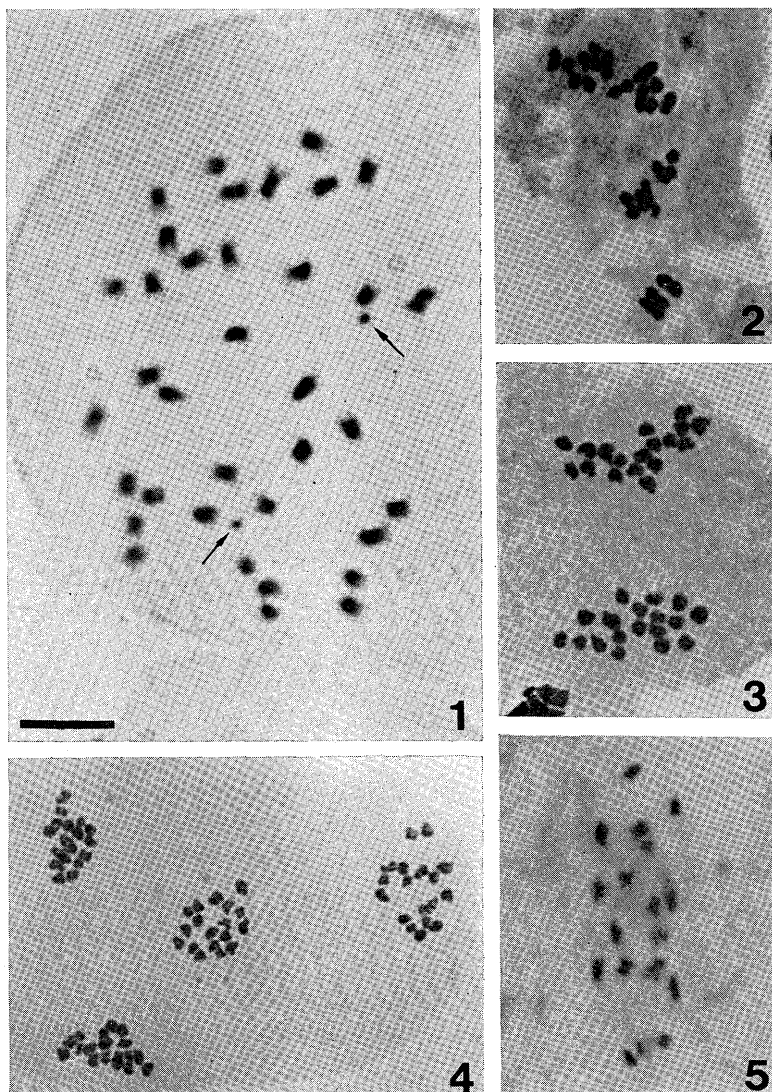
Species	Localities	n	2n	No. of plants examined
<i>D. dicarpon</i> (Miq.) W. T. Wang et Hsiao	Mt. Kosho, Fukuoka Pref.		36	5
	Mt. Kuju, Oita Pref.	18 _{II}	36	10
var. <i>decumbens</i> Tamura et Kosuge	Mt. Shiraiwa, Miyazaki Pref.		36	3
<i>D. hakonense</i> (Maekawa et Tuyama ex Ohwi)	Hakone, Kanagawa Pref.	18 _{II}	36	8
W. T. Wang et Hsiao	Mt. Hikane, Kanagawa Pref.	18 _{II}	36	6
<i>D. nipponicum</i> (Franch.) W. T. Wang et Hsiao	Mt. Shirouma, Niigata Pref.		36	5
	Mt. Yudono, Yamagata Pref.	18 _{II}	36	4
var. <i>sarmentosum</i> (Ohwi) Tamura et Kosuge	Mt. Hakodate, Shiga Pref.	18 _{II}	36	5
	Mt. Ogi, Hyogo Pref.		36	3
<i>D. numajirianum</i> (Makino) W. T. Wang et Hsiao	Mt. Obako, Nara Pref.		36	3
	Mt. Tamaki, Wakayama Pref.	18 _{II}	36	1
<i>D. pterigionocaudatum</i> (Koidz.) Tamura et	Hanase, Kyoto Pref.		36	5
Lauener	Mt. Hunakoshi, Hyogo Pref.	18 _{II}	36	5
<i>D. stoloniferum</i> (Maxim.) W. T. Wang et Hsiao	Mt. Fuji, Shizuoka Pref.		36	2
	Mt. Gyojagaeri, Nara Pref.	18 _{II}	36	4
	Mt. Mitsutoge, Yamanashi Pref.	18 _{II}	36	8
<i>D. trachyspermum</i> (Maxim.) W. T. Wang et Hsiao	Kibune, Kyoto Pref.	18 _{II}	36	15
	Mt. Buko, Saitama Pref.		36	5
	Mt. Kongo, Osaka Pref.	18 _{II}	36	14
	Mt. Otaki, Kagawa Pref.		36	9
	Mt. Ryozen, Shiga Pref.		36	6
<i>D. univalve</i> (Ohwi) Tamura et Lauener	Mt. Kongo, Osaka Pref.	18 _{II}	36	5
	Mt. Myojin, Kochi Pref.		36	8

Tab. 1. Voucher specimens are deposited in the herbaria of Kobe University and Kyoto University (KYO). The somatic chromosomes were observed in meristematic tissues at root tips. After preincubation with 2 mM 8-hydroxyquinoline for 2 hours at 15°C, they were fixed in the 2:1:1 mixture of ethyl alcohol, chloroform and acetic acid for more than 1 hour at 5°C. Then they were macerated with 1 N HCl for 15 sec. at 60°C and stained with 2% aceto-orcein applying the squash method. For observation of meiosis and microspore division, pollen mother cells (PMCs) and microspores were fixed by 45% acetic acid for more than 15 min. at 5°C, stained with 1% aceto-orcein and squashed.

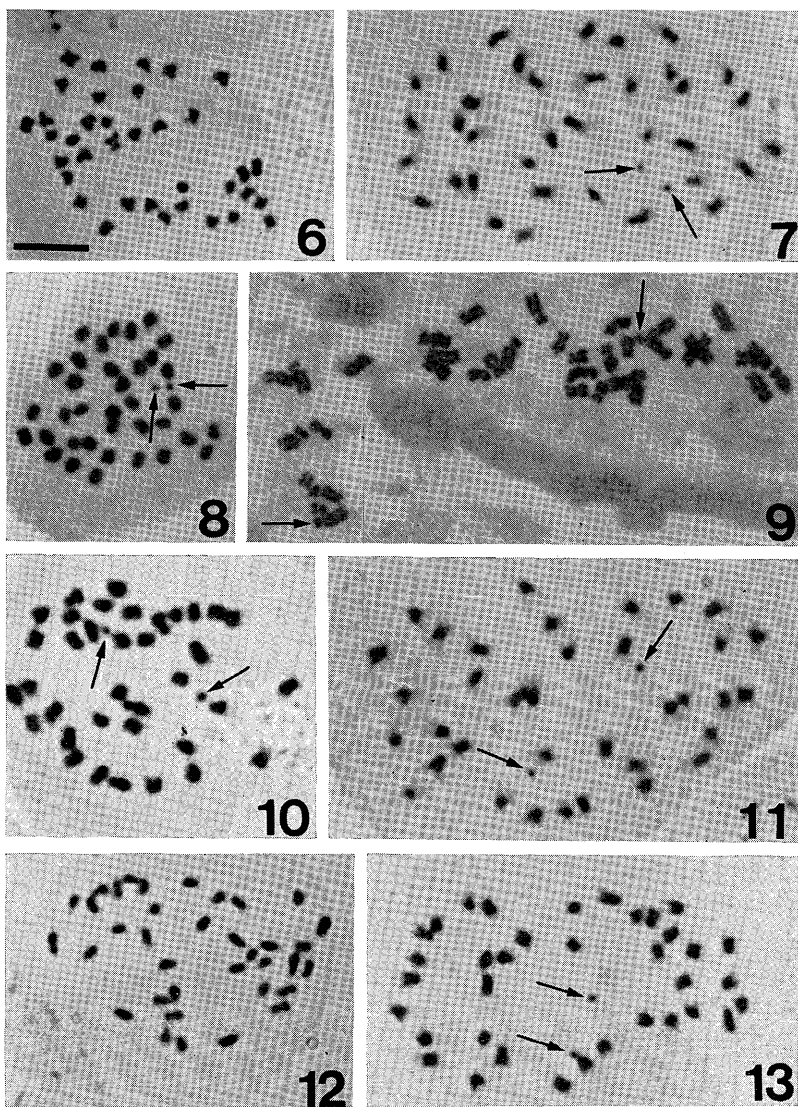
Observations and discussion All of the species observed showed the common karyological features. The chromosome number of 8 species and 2 varieties were $2n=36$ (Figs. 1, 6-13). The present results were different from those in the previous reports, i. e., $2n=35$ (Kurita 1954, 1956, 1966, 1967, Okada & Tamura 1979). The following description explains the karyological characters of *D. trachyspermum* as a representative of the specimens observed.

The somatic chromosome number of *D. trachyspermum* was 36 (Fig. 1). All of the 49 individuals at 5 different localities showed the same chromosome number, $2n=36$ (Tab. 1). Interphase nuclei and prophase chromosomes were categorized into T-type as reported by Kurita (1966). Chromosomes at metaphase were small, about 0.8-1.8 μm in length. This chromosome complement contained a pair of satellite chromosome which were obviously at the prometaphase (Fig. 1).

At metaphase I of PMCs (Fig. 2), 18 bivalents were observed in all of PMCs, but neither univalents nor multivalents were observed. At anaphase I, 18 chromosomes normally segregated toward the poles (Fig. 3). At anaphase II, each pole evenly contained 18 chromosomes (Fig. 4). In the subsequent microspore division, 18 chromosomes were observed at prometaphase (Fig. 5). Pollen grains consisted of two cells and showed high stainability by cotton blue. Open pollinated and bagged flowers resulted high seed sets (87%), while emasculated flowers did not (0%). At the end of flowering season, some accidental abnormal meiotic features appeared exceptionally, but these did not contribute to seed production. Almost all of seeds were generated at early or middle flowering season. On the contrary to the presumption that gametes develop abnormally, all of the evidences mentioned above indicate that this species produces normal gametes and fertilizes ordinarily. Actually normal pollen grains



Figs. 1-5. Photomicrographs of somatic chromosomes, phase of PMC divisions and microspore divisions of *Dichocarpum trachyspermum*. 1. Somatic prometaphase chromosomes. $2n=36$. Arrows indicate satellite chromosomes. 2. Metaphase I of PMCs, $18n$. 3. Anaphase I, 18 chromosomes segregate toward each pole. 4. Anaphase II, each pole contains 18 chromosomes. 5. Prometaphase of the first microspore division, $n=18$. Bar: $5\mu m$.



Figs. 6-13. Photomicrographs for somatic chromosomes of *Dichocarpum* species. 6. *D. dicarpon*, $2n=36$. 7. *D. dicarpon* var. *decumbens*, $2n=36$. 8. *D. hakonense*, $2n=36$. 9. *D. nipponicum*, $2n=36$. 10. *D. numajirianum*, $2n=36$. 11. *D. pterigonocaudatum*, $2n=36$. 12. *D. stoloniferum*, $2n=36$. 13. *D. univalve*, $2n=36$. Arrows indicate satellite chromosomes which cannot be identified in Figs. 6 and 12. Bar: $5\mu\text{m}$.

were reported in this genus by Ikuse (1954) and Kosuge & Tamura (1988).

Judging from the meiotic features, this chromosome complement, $2n=36$, did neither contain any accessory chromosomes nor additional chromosomes. The chromosome number, $2n=36$, is not a pentaploid level of $x=7$, but is presumed to be a tetraploid level of $x=9$ or a hexaploid level of $x=6$.

The genus *Dichocarpum* was divided into two sections mainly based on the shape of pedicel top (Wang & Hsiao 1964). Japanese species are classified into sect. *Hutchinsonia*, while the others belong to sect. *Dichocarpum* and are distributed in Himalayas, north Burma, Continental China and Taiwan. In this study, it is concluded that sect. *Hutchinsonia* is characterized cytologically as possessing $2n=36$.

On the other hand, Kurosawa (1971) reported $2n=ca. 28$ for *D. adiantifolium*, one of 10 species in sect. *Dichocarpum*, from Himalayas. This chromosome number differs from $2n=36$ of sect. *Hutchinsonia*. Thus, at the present, a real basic chromosome number this genus can not be defined until the chromosome numbers of sect. *Dichocarpum* are clarified.

The authors hope to express their sincere acknowledgment to Dr. M. Tamura, Prof. of Kobe Univ., for his kind encouragement and suggestions to this study.

References

- Gregory, W.C. 1941. Phylogenetic and cytological studies in the Ranunculaceae. Trans. Amer. Philos. Soc. 31: 443-521. Ikuse, M. 1954. On the pollen grains of *Isopyrum* (Ranunculaceae). Journ. Jap. Bot. 29: 331-332. Kosuge, K. & M. Tamura 1988. Notes on *Dichocarpum* Sect. *Hutchinsonia*. Acta Phytotax. Geobot. 39: 37-46. Kurita, M. 1954. On the chromosomes of some species in Ranunculaceae. Medic. Biol. 30: 185-187. ——— 1956. Cytological studies in Ranunculaceae, VI. On the chromosomes of *Aquilegia* and some other genera. Kromosomo 27: 939-941. ——— 1966. Chromosome studies in Ranunculaceae, XXIV. Mem. Ehime Univ. Sect. II, Ser. B, 5: 103-108. ——— 1967. ditto XXV. ibid. 5: 165-169. Kurosawa, S. 1971. Cytological studies on some Eastern Himalaya plants. In Hara, H. (ed.) The flora of Eastern Himalaya. Univ. Tokyo, Bull. 2: 658-670. Langlet, O. 1927. Beiträge zur Zytologie der Ranunculazeen. Svensk Bot. Tidskr. 21: 1-17. ——— 1932. Über Chromosomenverhältnisse und Systematik der Ranunculaceae. ibid. 26:

381-400. Okada, H. & M. Tamura 1979. Karyomorphology and relationship on the Ranunculaceae. Journ. Jap. Bot. 54: 65-77. Sorokin, H. 1929. Idiograms, nucleoli and satellites of certain Ranunculaceae. Amer. J. Bot. 16: 407-420. Tamura, M. 1966. Morphology, ecology and phylogeny of the Ranunculaceae VI. Sci. Rep. Osaka Univ. 15: 13-35. ——— 1968. ditto VIII. ibid. 17: 41-56. Taylor, R.L. & G.A. Mulligan 1968. Flora of the Queen Charlotte Islands part 2. Cytological aspects of the vascular plants. Research Branch Canada Depart. Agri. Monograph 4: 62-67. Wang, W.T. & P.K. Hsiao 1964. A new genus of Ranunculaceae—*Dichocarpum* W.T. Wang et Hsiao. Acta Phytotax. Sin. 9: 315-333, t. 32, 33.

* * * *

シロカネソウ属 *Dichocarpum* は約18種が東アジアの温帯より暖帯に分布する。多くの種は初め *Isopyrum* のもとに記載されたが、Wang と Hsiao (1964) により新しく独立した属に区別され、さらに花梗上部の形により2つの節に分けられた。日本に分布するものはシロカネソウ節 Sect. *Hutchinsonia* にまとめられ、このうち5種で染色体数、 $2n=35$ が報告された。染色体数は分類群を考える上で重要な特徴のひとつと見なされており、この属に近縁なチブシロカネソウ属、*Isopyrum*、ヒメウズ属、オダマキ属の染色体が $2n=14$ で基本数が $x=7$ であることより、この属の種は基本数が7の5倍体であると推定された (Kurita 1954, 1957, 1966)。しかしながら、今回、日本産のすべての種類、8種、2変種で染色体数 $2n=36$ を観察した。また花粉母細胞の減数分裂において、18個の2価染色体が見られ、1価染色体あるいは多価染色体は認められず、減数分裂は正常であることより、5倍体ではないことがわかった。従って、シロカネソウ属シロカネソウ節における基本数は $x=6$ あるいは $x=9$ と推定され、近縁な属と基本数が異なる。

□Gulden, G. & K.M. Jenssen: **Arctic and alpine fungi-2** 58 pp. 1988. Soppkon-sulentent, Wesselsgt. 3, 0165 Oslo 1, Norway. 極地および高山のマツタケ目菌類についてルーズリーフ方式で、一葉に一種ずつ、カラー写真と顕微鏡スケッチと記載と分布・生態などのメモを載せる。第1巻 (Gulden, Jenssen & Stordal 1985) で主に南ノルウェーの山岳地帯のものを13属25種 (1新組合わせ)、本冊ではスビッツベルゲンのもので1巻にない13属25種 (1新種, 1新組合わせ) を取扱っている。写真はボタニカル・アートの伝統を感じさせ、羨ましさを感じる。 (三浦宏一郎)